

## APPENDIX II

### CLEAN VERSION OF THE ENTIRE SET OF PENDING CLAIMS AS AMENDED IN THIS COMMUNICATION

The following is a list of the Claims as they would appear following entry of this amendment.

49. A method for preparing an evolved microorganism comprising the steps of:
- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein said at least one heterologous protein is an enzyme;
  - b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type; and
  - c) restoring said evolved microorganism to a wild type mutation rate.
50. The method of Claim 49, wherein said at least one heterologous protein is a hydrolase.
51. The method of Claim 50, wherein said hydrolase is selected from the group consisting of proteases, esterases, lipases, phenol oxidase, permeases, amylases, pullulanases, cellulases, glucose isomerase, laccases, and protein disulfide isomerases.
52. The method of Claim 49, wherein said microorganism comprises at least one copy of said mutator gene in its chromosome and said step of restoring said evolved microorganism to wild-type mutation rate comprises excision of said mutator gene.
53. The method of Claim 52, wherein said mutator gene comprises at least one gene selected from the group consisting of *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, *mutU*, *mutD* mutations, *mutT* mutations, *mutY* mutations, *mutM* mutations, *mutH* mutations, *mutL*

mutations, *mutS* mutations, *mutU* mutations, and homologues of *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, and *mutU*.

54. The method of Claim 52, wherein said mutator gene comprises *mutD* mutations selected from the group of *mutD* mutations set forth in Table 1.

55. A method for preparing an evolved microorganism comprising the steps of:
- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein said at least one heterologous protein is an enzyme necessary for an enzymatic pathway;
  - b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type; and
  - c) restoring said evolved microorganism to a wild type mutation rate.

56. The method of Claim 55, wherein said enzyme is selected from the group consisting of reductases and dehydrogenases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of ascorbic acid or ascorbic acid intermediates.

57. The method of Claim 55, wherein said enzyme is selected from the group consisting of glycerol dehydratase and 1,3-propanediol dehydrogenase, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of 1,3-propanediol, 1,3-propanediol precursors, and 1,3-propanediol derivatives.

58. The method of Claim 55, wherein said enzyme is selected from the group consisting of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of glycerol and glycerol derivatives.